RotaTeq®
(Rotavirus Vaccine, Live, Oral, Pentavalent)

Update on Porcine Circovirus (PCV)

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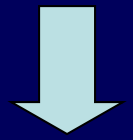
Key Tools and Approach Applied in the PCV Investigation for RotaTeq®

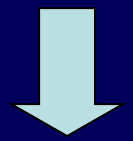
Goal: develop the analytical tools capable of detecting very low levels of PCV DNA in RotaTeq® and systematically demonstrate the absence of infectious PCV

- **Quantitative PCR**: Presence and quantity of short fragments of PCV1 and PCV2 DNA (<100 bp)



• if detected

- **Endpoint PCR**: Presence of longer fragments of PCV1 and PCV2 DNA (~800 bp) which could be associated with intact virus particles



• if detected

- **In vitro infectivity assay**: Presence of infectious PCV1 and PCV2 via infectivity assay in permissive cell line using QPCR as endpoint detection method

Establishing appropriate assay conditions was critical to the ability to detect presence of viral DNA and infectious virus

Is PCV Present in RotaTeq®?

Evaluation of Final Container and Bulk Lots

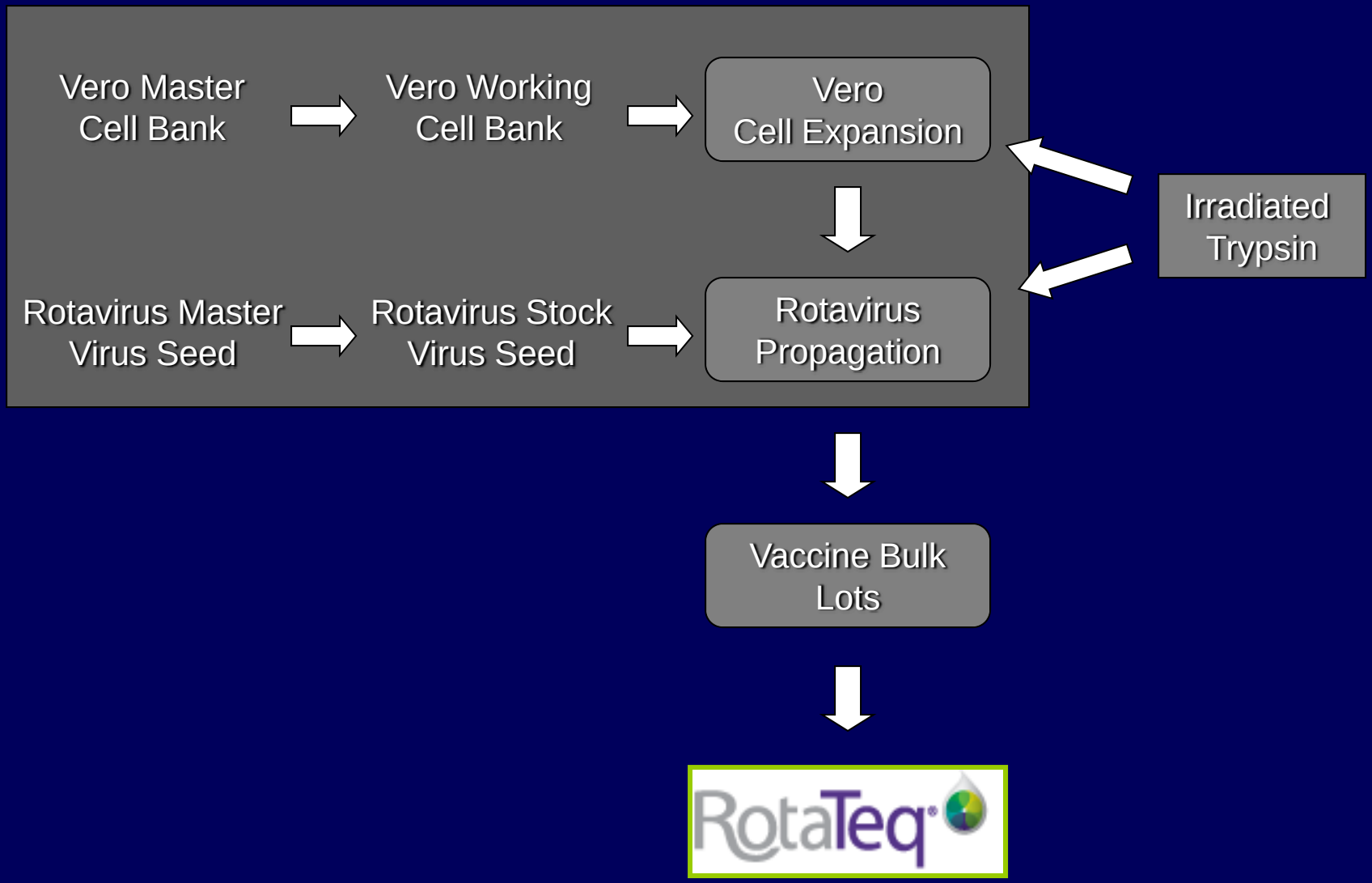
Material Tested	QPCR (copies/ml) (<100 bp)		Endpoint PCR (~800 bp)		Infectivity Assay 28 Day Test
	PCV1 DNA	PCV2 DNA	PCV1 DNA	PCV2 DNA	Infectious PCV
RotaTeq® **3 Final Container Lots**	1.4 to 1.7 x10^3 No differentiation between PCV1 & PCV2		N/A	N/A	N/A
31 Rotavirus Bulks **(Commercial facility)**	<LOD	3.7 to 7.8x10^3	Negative	Positive* (11) Negative (20)	Negative
Rotavirus Clinical Bulks **(REST – pilot facility)**	Negative	Negative	N/A	N/A	N/A

N/A – not applicable (testing not performed)
<LOD = Below limit of detection
* at limit of detection (LOD)

Conclusions:

- PCV2 DNA is detected in Rotavirus bulk lots manufactured in the commercial facility
- Infectious PCV is NOT detected in any bulk lots

What is the Source of PCV DNA?
Evaluation of Process Inputs



What is the Source of PCV DNA?
Evaluation of Process Inputs

Material Tested	**QPCR** (copies/ml) (<100 bp)		**Endpoint PCR** (~800 bp)		**Infectivity Assay 28 Day Test**
	PCV1 DNA	PCV2 DNA	PCV1 DNA	PCV2 DNA	Infectious PCV
Vero Master Cell Bank	Negative	Negative	Negative	Negative	N/A
Vero Working Cell Bank	Negative	<LOD	Negative	Negative	N/A
Rotavirus Master Seeds	Negative or <LOD	Negative or <LOD	N/A	Negative	N/A
Rotavirus Stock Seeds	Negative	<LOD	N/A	Negative	N/A
Irradiated Trypsin	<LOD	**Positive**	N/A	Negative	Negative

N/A – not applicable (testing not performed)
<LOD = Below limit of detection

Are Antibody Responses to PCV2 Detected in Serum Samples of Vaccine Recipients?

- Vaccine Recipients Tested:
  - Clinical consistency lot vaccine recipients
    - Manufactured using bulk lots shown to contain low levels of PCV2 DNA
    - All available serum samples tested
  - Pre & post dose 3 sera available for testing
- 79 paired (158 total) serum samples tested by ELISA
  - 79 samples pre dose 1; 79 samples 42 days post dose 3

Treatment Group	Interval	Samples Tested	PCV2 Antibody Results
Placebo	Pre	13	negative
Placebo	Post	13	negative
Vaccine	Pre	66	negative
Vaccine	Post	66	negative

Conclusion:

- All samples were seronegative for PCV2

Summary of PCV Investigation of RotaTeq®

PCV1 DNA Fragments

- Below limit of detection (<LOD) in bulk lots of RotaTeq®

PCV2 DNA Fragments

- Low levels detected in bulk lots of RotaTeq®
  - Trypsin has been confirmed as the source of PCV2 DNA fragments

Infectious PCV

- NOT detected in bulk lots of RotaTeq® or in process inputs associated with the manufacture of RotaTeq®

PCV2 Antibodies in Serum Samples

- NOT detected in serum samples of vaccine recipients who received clinical material that contained low levels of PCV2 DNA fragments

All key aspects of the PCV investigation of RotaTeq® have been completed and the findings are being communicated to regulatory agencies worldwide